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IMPROVEMENTS RELATING TO THE PRODUCTION OF MONOCLONAL ANTIBODIES

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A process for producing antibodies against a selected target epitope comprising inoculating a host animal with transfected cells syngeneic with the host animal capable of expressing a polypeptide and recovering antibodies from the serum or other body fluid of the animal, the polypeptide comprising a portion comprising the same amino acid sequence as the target epitope and at least one flanking portion having the same amino acid sequence as the corresponding flanking portion of a host animal protein homologous with the protein which bears the target epitope.

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IMPROVEMENTS RELATING TO THE PRODUCTION OF MONOCLONAL ANTIBODIES

The present invention relates to a process for producing monoclonal antibodies.

Monoclonal antibodies (MAb's) were first described by Kohler and Milstein [Nature, 256, 495-497, (1975)]. Since then MAb's have found widespread use in research and are increasingly used in therapy and diagnosis. The now almost routine process for obtaining MAb's against a particular immunogen (antigen or hapten) involves inoculating animals with the immunogen, obtaining antibody-producing cells from the inoculated animal and immortalising these cells usually by fusion with immortal cells such as myeloma cells derived from the same species, to form an antibody producing hybridoma cell line.

At this stage of the process it is necessary to screen clones of the hybridoma cells to identify those producing antibodies against the original immunogen. Usually the antibody producing cells from the inoculated animal are also screened to identify suitable candidates for immortalisation. Even when highly purified immunogens are employed it is usually found that only a very small proportion of the hybridoma cells produce the desired antibodies. Sometimes there are none at all. The uncertainty of ever obtaining a satisfactory antibody, and the delays involved in the screening process are considerable drawbacks for which there has, until recently, been no solution.

It has recently been shown that the production of antibodies against a particular polypeptide can be greatly increased by inoculating the animals with substantially genetically identical cells, which cells have been transfected with DNA coding for the polypeptide.

It is believed that by using cells from a

syngeneic donor animal, the differences between the transfected cells and the host animals own cells that can be detected and acted upon by the host animal's immune system are minimised. Thus any immune response is more likely to be directed against the foreign polypeptide produced by the transfected cells by expression of the DNA sequence.

Large polypeptides, especially mature proteins, usually present a number of different antigenic sites or epitopes. It is often desired to raise antibodies against a single specific epitope and this has, in the past, involved elucidation of the amino acid sequence of the epitope, synthesis of an oligopeptide analogue of the epitope and use of the oligopeptide as an immunogen. Oligopeptides are, however, poor mimics of epitopes as they are free to adopt conformations which are different from the native conformation of the epitope. Thus the antibodies raised against the oligopeptides, whilst capable of specific reaction with the oligopeptide often do not react specifically with the desired epitope or react only weakly with it.

An improved method for producing antibodies against a particular epitope has now been developed in which the peptide analogues of the epitope are constrained to match the conformation of the epitope and antibody production is directed against the thus-constrained analogue.

Accordingly the present invention provides a process for producing antibodies against a selected target epitope comprising inoculating a host animal with transfected cells syngeneic with the host animal capable of expressing a polypeptide and recovering antibodies from the serum or other body fluid of the animal, wherein the polypeptide comprises a portion comprising the same amino acid sequence as the target epitope and at least one flanking portion having the same amino acid sequence as the

corresponding flanking portion of a host animal protein homologous with the protein which bears the target epitope. Such polypeptides will hereafter be referred to as foreign-epitope containing polypeptides or "FECP's".

The present invention further provides a process for producing a cell capable of secreting antibodies against a selected target epitope comprising inoculating a host animal with transfected cells syngeneic with the host animal capable of expressing a FECP and removing antibody-secreting cells from the animal. The cells may be capable of secreting antibodies in vitro, for instance under appropriate cell culture conditions, and/or in vivo, for instance by growth in ascites.

As used herein the term "animal" refers to any animal capable of producing antibodies for instance mammals and birds and includes humans.

As used herein the term "antibody" includes polyclonal and monoclonal antibodies and fragments thereof such as the $F(ab)_2$ fragment unless the context requires otherwise.

Transfected cells are cells containing a DNA vector containing a sequence encoding the FECP and are derived from an animal (hereafter the "donor animal") syngeneic with the animal (hereafter the "host animal") which is inoculated with the transfected cells. Without wishing to be bound by a particular theory, it is believed that any immune response is likely to be directed against the foreign portion (corresponding to the target epitope) of the FECP produced by the transfected cells by expression of the DNA sequence. The flanking portions of host protein constrain the foreign portion to adopt a conformation closely resembling the conformation of the target epitope and spurious antibody production is therefore minimised.

To maximise the directing effect on the immune system of the host animal it is desirable that the host and donor animals are substantially genetically identical and

usually they will be from a well established inbred strain such as are well known in the art. Use of monozygotic donor and host animals whether or not from an inbred strain, or even cells from the host animal itself, is contemplated.

As the skilled person will appreciate epitopes may be formed by a single contiguous portion of a polypeptide and can be as small as two or three amino acid residues in length. Alternatively an epitope may comprise a number of amino acid residues which whilst not adjacent in terms of the primary protein structure, are bought into close proximity to one another by folding of the polypeptide backbone according to the secondary or tertiary structure of the protein.

The foreign portion of the FECP expressed by the transfected cells may therefore be of any suitable length required to mimic the target epitope and could, for instance, comprise two or more, preferably five or more amino acid residues. The maximum length of the foreign portion will depend on the nature of the epitope and the foreign portion could for instance correspond to at least a complete domain and may include more than one domain or parts of more than one domain of the protein bearing the target epitope.

Some epitopes involve non-peptide moieties, for instance mono-, oligo- or polysaccharides and in such cases it will be necessary for the transfected cell to be capable of processing the FECP in order to provide the appropriate additional moieties to form the complete epitope.

The FECP expressed by the transfected cells will also comprise at least one flanking sequence of the homologous host protein and preferably comprises portions, at both the N and C termini of the foreign portion, having sequences identical to the corresponding portions of the homologous host protein. The flanking portions will be sufficiently large to create a model of the native

environment of the target epitope. When the target epitope is an oligopeptide the flanking portion(s) may be simply the remainder of a corresponding domain of the host protein though ideally they will correspond with the whole of the rest of the host protein. On the other hand, if the foreign portion constitutes an entire domain, it may be necessary for the flanking portion(s) to comprise at least several domains and possibly the entire remainder of the host protein.

Where the target epitope appears on a multi-chain or multi-sub unit protein it may be necessary for the transfected cells to express all the chains or all the sub-units and this may be achieved either by selecting cells for transfection which are already capable of expressing the necessary additional chains or sub-units and transfecting only with DNA encoding a FECP corresponding to the single chain or sub-unit of interest, or it may be necessary to transfect with DNA encoding the other components as well. In the former case, suppressing the expression of the host homologous protein chain or sub-unit may also be advantageous.

In another aspect the invention provides a process for producing a cell capable of secreting antibodies against a target epitope comprising removing antibody-secreting cells from a host animal inoculated with transfected syngeneic cells capable of expressing a FECP.

In a further aspect the invention provides a cell capable of secreting antibodies against a selected polypeptide which cell has been removed from a host animal inoculated with transfected syngeneic cells capable of expresssing a FECP.

It may be convenient at this stage to screen the population of antibody-secreting cells obtained from the host animal in order to select those secreting antibodies against the target epitope.

The cells are useful particularly for immortalisation, for instance by fusion with immortal cells, in order to form an immortal cell line capable of secreting antibodies (hereafter monoclonal antibodies or MAb's) against the target epitope. When immortalisation is by fusion with an immortal cell the product is a hybridoma.

Accordingly, the invention also provides a process for producing an immortal cell capable of secreting antibody against a target epitope comprising immortalising an antibody-secreting animal cell, as hereinbefore defined.

Immortal cells (which cells are capable of repeated cell division under appropriate culture conditions) suitable for fusion are well known. The immortal cell may be of the same inbred strain as the antibody-secreting cells and usually they will be of the same species as the antibody-secreting cells from the host animal but this is not essential. Particularly convenient immortal cells are myeloma cells.

After immortalisation it may be convenient to screen the resulting population of immortal cells to select those secreting antibodies against the target epitope.

The present invention also provides an immortalised cell, capable of secreting antibodies against a target epitope which cell comprises an immortalised antibody-producing cell as hereinbefore defined especially a hybridoma, or is a descendant of such a cell.

Immortalised cells (e.g. hybridoma cells) according to the present invention are useful for producing MAb's against the target epitope.

The present invention also provides a process for producing monoclonal antibodies against a target epitope comprising culturing an immortalised cell, as hereinbefore defined or a descendant of such a cell, and recovering the monoclonal antibodies.

The invention further provides monoclonal antibodies against a target epitope which antibodies are

obtained from growth in tissue culture or ascites of an immortal hybrid cell as hereinbefore defined.

In order to obtain antibody producing cells, immortal cells and MAb's against a target peptide epitope according to the present invention, DNA coding for the homologous host protein or an appropriate portion thereof is identified and obtained from a natural source or produced by partial or total synthesis. The DNA is modified by conventional techniques such that a foreign portion is introduced encoding the amino acid sequence of the target epitope. Techniques for obtaining natural or artificial DNA encoding a particular polypeptide are well known in the field of genetic engineering and are described in Maniatis, T., Fritsch, E and Sambrook J. (1982) Molecular Cloning, a Laboratory Manual, published by Cold Spring Harbor Laboratory Press NY. Modification of the genomic or cDNA may be by restriction endonuclease excision of a fragment and ligation of a corresponding fragment including a sequence encoding the foreign portion of the FECP to be expressed, such as in "exon shuffling" techniques. Alternatively the DNA may be modified by site specific mutagenesis such that a sequence encoding the foreign portion of the FECP is generated in vitro. The vector is constructed by conventional methods to ensure expression in the cells to be transfected and optionally to provide regulatory and marker sequences enabling control of expression and identification of the DNA and transfectant cells, (Maniatis loc. cit.). Selective markers which are particularly suitable in the present invention include drug resistance markers such as the thymidine kinase (tK) gene for tK^- cells. (Szybalska, E and Szybalski W. (1962) Proc. Natl. Acad Sci., USA, 42, 2026).

Suitably, promoter sequences may be inserted in the vector to increase expression of the FECP in the transfectant cells (Subramani, S., Mullingan, R.C. and Berg, P. (1981) Mol. Cell. Biol. 1, 854-864).

The cells to be transfected, i.e. the cells into which the vector is to be inserted (hereafter "donor cells"), are obtained from donor animals, such as rodents, especially rats and mice, and primates, for instance monkeys; most preferably mouse cells are used.

The donor cells must be capable, once transfected, of expressing the FECP and, where necessary, of any required post-transcriptional and/or post translational processing of the FECP. Preferably the donor cells and the optionally modified DNA are chosen such that the FECP appears extracellularly or at the cell surface although it is also possible to use FECP's which are only expressed intracellularly in the present invention. For intracellularly expressed FECP's, it is preferred that the donor cells are of a type not normally found in the peritoneum or circulating in the bloodstream of the host animals.

Transfection of donor cells is achieved by conventional methods such as using calcium phosphate (Austin, P., Trowsdale, J., Rudd, C., Bodmer, W., Feldmann, M. and Lamb, J. (1985) Nature, 313, 1-4) or by the electroporation technique (Neumann, E., Schaefer- Ridder, M., Wang, Y. and Hofschneider, P.H. (1982) EMBO J, 1, 841-845).

In further embodiments the invention provides nucleic acid fragments encoding FECP's as hereinbefore defined including genomic DNA comprising introns and exons, RNA transcribed therefrom, mRNA corresponding to coding portions thereof and cDNA reverse transcribed therefrom, as well as expression vectors comprising such DNA and cells transfected with such expression vectors.

After transfection it may be convenient to screen the cells, or clones or sub-clones thereof, to select those capable of expressing the FECP. With cell surface-expressed gene products the use of fluorescent antibodies and a fluorescence activated cell sorter

is particularly convenient (Austin loc. cit.) but other techniques are available for screening and selection.

Transfectant cells, optionally after selection, may be treated to enhance the expression of the gene product. Various known enhancement methods can be used including treatment with sodium butyrate (Gorman, C.M. and Howard, B.H. (1983) Nucleic Acids Research, 11, 7631-7648) or interferon (Balkwill, F.R., Stevens, M.H., Griffin, D.B., Thomas, J.A. and Bodmer, J.G. (1987) Eur. J. Cancer Clin, Oncol 23, 101-106. The enhancement method will be selected as appropriate to the type of donor cells, the polypeptide to be expressed and the promoter used.

The host animal or animals are inoculated with transfected cells, preferably by intraperitoneal or intravenous injection of transfected cells in a suitable medium. Preferably the inoculation is repeated at intervals and the animals' antibody titre is monitored to ascertain that the desired immune response occurs. The route chosen for administration and the inoculation regime are preferably selected in order to favour production of the desired class of antibodies, eg production of cells secreting IgM antibodies is favoured by a short interval between inoculation and recovery of antibody-secreting cells, whereas production of cells secreting IgG antibodies is favoured by a long interval between repeated inoculation and eventual recovery of the antibody-secreting cells.

On completion of the inoculation regime antibody -secreting cells (hereafter "host cells"), eg, spleen cells, are taken from the inoculated host animal(s) and maintained in an appropriate medium. Conventional methods may be used for obtaining and maintaining the host cells.

Immortalisation may be by any of the known methods but fusion with an immortal cell to form a hybridoma is perhaps the most convenient method.

To produce hybridoma cells, host cells are fused with immortal cells, preferably myeloma cells from animals

preferably of the same species. Fusion is achieved by the method of Kohler and Milstein loc. cit. and by variations thereon as described in the literature.

Antibodies produced by the thus-produced the immortalised cells are screened by conventional techniques such as described in Lansdorp, P.M., Astaldi, G.C.B., Ooserhof, F., Janssen, M.C. and Zeijlemaker, W.P. (1980) J. Immunol. Methods, 39, 393; Hawkes, R., Niday, E. and Gordon J. (1982) Analyt. Biochem. 119, 142-147; Epenetos, A.A., Bobrow, L.G., Adams, T.E., Collins, C.M., Isaacson, P.G., Bodmer, W.F. (1985) J. Clin. Path. 38, 12-17; Bodmer J.G., Heyes. J.M. and Lindsay, J. (1984) Histocompatibility Testing edited by E.D. Albert. Springer Verlag 1984, 432-438, and Towbin, H., Staehelin, R. and Gordon, J. (1979) Proc. Nat. Acad. Sci. 76, 4350-4354) to identify those cells producing MAb's against the target peptide. Such cells may be multiplied and cultured to produce MAb's according to the invention in large quantities. Suitable culturing techniques and ascites growth conditions are well known and are chosen as appropriate to the particular immortalised cell line involved and the MAb's to be produced.

Accordingly, in a particular embodiment the present invention provides a process for producing monoclonal antibodies against a target epitope of a protein comprising the steps of (a) transfecting cells from a donor animal with a vector containing a DNA sequence encoding for a FECP and capable of expression in the transfected cells,

(b) inoculating an animal syngeneic with the donor animal with the transfectant cells.

(c) fusing immortal cells with cells from the inoculated animal which produce antibody against the target epitope and

(d) culturing the immortal hybrid cells thus produced.

The present invention may be applied to any

peptide epitope of interest, for instance antigens, toxins or protein hormones comprising polypeptides for which DNA may be obtained or synthesised. The polypeptide may be associated with disease processes or they may be non-therapeutic, non-disease polypeptides. If the polypeptide is correctly processed by the transfectant cells the present invention may also be used in obtaining antibody producing cells or immortal hybrid cells producing antibodies against non-peptide portions of the final product, e.g., against the carbohydrate portion of a glycoprotein. One particular application of the present invention is in the production of MAb's for tissue typing by using DNA sequences coding for portions of the HLA regions of the human genome.

The present invention further provides polyclonal and monoclonal antibodies produced by the processes defined above. In particular aspects there are provided polyclonal and monoclonal antibodies and fragments thereof against the peptide epitopes of polypeptides encoded by a series of variable and constant regions of the major histocompatibility complex of a mammalian genome, such as antibodies against the product of a polymorphic gene which antibodies are capable of recognising the products of the equivalent genes of other individuals (frequently known as "polymorphic antibodies"). This technique may be applied to the production of monoclonal antibodies which are monospecific for the allelic products of the HLA DP, DQ and DR regions of the human genome such as DR7. Certain of these polypeptides are apparently of only low immunogenicity and it has previously been difficult or impossible to raise antibodies against them whereas this may be achieved according to the processes of the present invention.

Antibodies, whether polyclonal or monoclonal, against disease-associated polypeptide may be used in therapeutic methods for treating humans and animals, for

instance in the form of vaccines for passive immunisation. The invention therefore also provides pharmaceutical compositions comprising an antibody of the invention and a suitable carrier. For injection the compositions may be in the form of aqueous sterile, non-pyrogenic solutions optionally containing buffers, antioxidants, biocides such as antibacterials and antifungals and agents to adjust the tonicity. Alternatively the compositions may be provided as dry powders, optionally containing excipients such as those mentioned above, for reconstitution by addition of water for injection. Dosage rates will depend on the age, weight, size, sex and general health of the patient and will be sufficient to achieve appropriate levels of circulating antibodies without undesirable or intolerable side effects. The antibodies may also be used in immunological procedures such as diagnostic and other immunoassay procedures including tissue typing using antibodies against proteins encoded by the HLA region of the human genome which also form an aspect of the invention. Suitable procedures are well known in the art.

The invention is therefore useful in providing methods and materials for use in medicine including therapeutic and diagnostic uses. It can be used to produce antibodies against proteins including glycoproteins. Particular proteins of interest are those of the major histocompatibility complex of the mammalian genome and oncogenes, growth factor and other receptors and enzymes.

In a particular embodiment the invention may be used to generate antibodies directed toward desired epitopes of cell surface receptor proteins such as the CD4 molecule on human cells, which act as a receptor for the Aids virus. There have been difficulties in raising monoclonal antibodies to the particular epitopes or regions of the CD4 molecule which are involved in binding the HIV virus, thought to be in the portion of about 100 amino acids at the N-terminal, although some have, fortuitously, been generated. With the present invention it is possible to insert an FECP for specific regions of the human CD4

molecule into the mouse or other mammalian homologous cell surface receptor, and thereby generate antibodies directed at desired portions of the CD4 molecule. The process of the invention is also used in the identification of unknown protein gene products where the gene or a portion thereof has been identified and can be inserted into a suitable vector for expression in transfected cells since the antibodies produced by the present invention can be used to isolate and identify the gene product from its natural evironment where it is expressed as a mature protein.

The invention will now be illustrated by an Example and with reference to the accompanying drawings in which:

Fig. 1 shows schematically the construction of human-mouse hybrid gene DR4DW4/E^d and a vector containing the gene, in accordance with the invention.

Fig. 2 shows the construction of the pJ4-DR4Dw15/E^d hybrid gene; the 0.4K DR beta Wa Eco RI-Pst I fragment containing a sequence encoding the first 79 amino acid residues of the DR4Dw15 beta gene is ligated with the 0.8K Pst I - Eco RI fragment of the IE^d beta gene encoding the residues 80 - 237 of the murine E^d beta chain into the pJ4 expression vector at its Eco RI site. The pJ4 vector uses the Moloney murine leukaemia virus long terminal repeat sequence as the promotor for inserted cDNA. Purified DR beta Wa cDNA was obtained by conventional methods and provided by H. Ikeda. pJ4 was provided by J. Morgenstern.

Fig. 3 compares the sequences of the DR4Dw4/E^d and DR4Dw15/E^d constructs with that of IE^d (9). DR4 sequences are given in ref 10.

EXAMPLE 1

(a) Construction of a human-mouse hybrid gene DR4Dw4/E^d

In the following description all DNA manipulations (e.g. restriction endonuclease digestions, ligation, transformation, cloning and isolation of plasmid DNA, purification and electrophoresis of inserts) was by standard methodology such as is described in Maniatis et al (loc. cit.). Plasmid cloning was performed in E. coli MC 1061. Competent MC 1061 cells were made and transformed by standard techniques (Maniatis et al). Restriction enzymes and vector pUC-19 were from New England Biolabs, 32 Tozer Road, Beverley, M.A., USA. Ligase was from Anglian Biotechnology.

Plasmids p1201-1 and p713-2 were provided by G. Andersson of the Department of Cell Research, The Wallenberg Laboratory, University of Uppsala, Sweden having been produced from a library made from a homozygous DR4Dw4 individual.

Cosmid clone 24.2 in vector pTL5 was obtained from a Balb/c library (Steinmetz et al. (1982) Cell, 28, 429; Steinmetz et al, (1982) Nature, 300 : 35)

As shown schematically in Fig. 1, the hybrid gene comprises exons I and II of human DR4Dw4 beta chain and exons III to VI of murine 1-E^d beta chain(Fig. 1a). It is constructed as follows;

Plasmid p1201-1, a 4.6k plasmid comprising the pUC-19 vector and a 1.9k genomic insert, is digested with Eco RI and Bam HI to afford a 1.7k Eco RI-Bam HI fragment containing the human promotor and signal sequence, exon I (Fig. 1b).

Plasmid p713-2, a 6.1k plasmid comprising the pUC-19 vector and two copies of a 1.7k genomic insert is digested with Bam HI to afford a 1.7k Bam HI-Bam HI fragment containing human exon II (Fig. 1c).

Plasmid pTL5-24.2, a 43k plasmid comprising the

pTL5 vector and cosmid 24.2 is digested with Eco RI and the digest subjected to electrophoresis on agarose and the top (most mobile) band was purified. This Eco RI - Eco RI fragment containing the pTL5 vector and murine exons III-VI was circularised via the Eco RI sticky ends to afford a 19k plasmid pTL5-24.2t (Fig. 1d). This was in turn digested with Bam HI and Kpn I affording a 2.9k Bam HI-Kpn I fragment containing murine exons III-VI of the mouse gene (Fig. 1d).

The 1.7k Eco RI-Bam HI p1201-1 fragment (Fig. 1b), Bam HI-Bam HI p713-2 fragment (Fig. 1c) and Bam HI-Kpn I pTL5-24.2t fragment (Fig. 1d) were ligated into the larger fragment of an Eco RI and Kpn I digest of vector pUC-19 to give pUC-DR4Dw4/E^d (Fig. 1e).

Following litigation, MC1061 cells were transformed with the hybrid gene by standard techniques (Maniatis et al.) and colonies were plated out on LB Ampicillin agar plates. A number of colonies were picked at random and used for small scale preparation of plasmid DNA by standard techniques (Maniatis et al.). Restriction endonuclease mapping of these cells was performed to confirm the presence and orientation of the inserts. Colonies with correctly oriented inserts were selected for further purification of the hybrid gene.

(b) Transfection of Mouse Cells

(i) LtK^- cells were transfected using the calcium phosphate coprecipitation method (Van Pel, A., De Plaen, E. and Boon T. (1985) Somatic Cell & Molecular Genetics 11, 467-475). The transfected cells were assayed for expression of the genes using an indirect immunofluorescent assay (Maddox, J.F. et al., (1985) Immunology 55, 739) on the third day following transfection. The monoclonal antibody 14.4.4s (Ozato , K. et al., (1980) J. Immunol. 124, 533) which binds to the murine I-E alpha chain was used to detect the complex.

FITC-RAM Ig was purchased from DAKO.

(ii) The P815 HTR (H-2d) mastocytoma cell line (T. Boon) was cotransfected with the pUC-DR4Dw4/E^d constuct described above together with the murine IE alpha7 gene (obtained from R. Germain) in pJ4 expression vector (pJ4-E alpha 7) and the neo gene in pSV2 expression vector (pSV2-neo) using calcium-phosphate coprecipitated DNA as described by Van Pel et al (1985). Cells were grown in E4 medium supplemented with 10% FCS, L-glutamine and penicillin-streptomycin in a humidified incubator with 10% carbon dioxide and G418 was added to the medium the day after the transfection to select for cells which had taken up DNA. 8 days after transfection the G418 concentration of the medium was reduced to 0.5 mg.ml. MHC II positive cells were sorted for on a FACS following indirect immunofluorescence using 14.4.4S and designated P815 1.

(c) Production of Monoclonal Antibodies

DBA/2 mice were immunised intraperitoneally with 10,000,000 live cells. One monoclonal antibody, X1.26, which reacts with Class II was recovered from this fusion.

EXAMPLE 2

A hybrid human-mouse gene designated DR4Dw15/E^d was constructed as indicated in Fig. 2 in expression vector pJ4. P815HTR cells were cotransfected with pJ4-DR4Dw15/E^d, pJ4-E alpha7 and pSV2-neo as in Example 1 and cells expressing the complex of hybrid alpha chain and murine beta chain at the cell surface were selected and designated P815 5.

EXAMPLE 3

The reactivity of a panel of anti-human MHC class II anitbodies was tested against the transfected P815#1 and P815 5 cells and against control cells (P815#3) transfected

with murine $1E^d$ gene place of the constructs of Examples 1 and 2.

The properties of the transfectants are shown in Table I and the reactivities of the antibodies are shown in Table II.

As most of the monoclonal antibodies which bind to human MHC class II molecules have been raised in Balb/c mice it is unlikely that these antibodies will bind to the murine $1E^d$ molecule and indeed none of those tested bound to $1E^d$.

TABLE 1

Properties of transfectants expressing hybrid human-murine MHC II molecules

	P815#1	P815#3	P815#5
alpha chain	E alpha^7	E alpha^7	E alpha^7
beta chain	DR4Dw4/E^d	E^d	DR4Dw15^d
resistance gene	neo	neo	neo
selection in	G418	G418	G418
lavel vs Priess*	1/3		1/45

* The relative levels of surface expression were compared following FACS analysis of cells stained with TDR31.1 in an indirect immunofluorescent assay (12).

TABLE II

Reactivity of anti-MHC class II antibodies vs transfectants

Transfectant Antibody	P815#1	P815#5	P815#3	P815
TAL185(13)	-	-	-	-
14.4.4S(14)	+	+	+	-
TDR31.1(15)	+	+	-	-
87/21(16)	-	-	-	-
CA2(17)	-	-	-	-
DA2(18)	-	-	-	-
M1.6	+	+	-	-
M1.18	+	+	-	-
M2.2	-	-	-	-
M2.27	+*	+	-	-
M2.69	+*	+	-	-
M2.72	+	+	-	-
M2.86	-	-	-	-
X1.26	+	-	-	-

*M2.27 and M2.69 bound much less strongly to P815 1 than to P815 5. Monoclonal antibodies M1.6, M1.18, M2.2, M.2.27, M2.69, M2.72, M2.86 and X1.26 are hither to unpublished antibodies which bind to MHC class II molecules and were made in the Tissue Antigen Laboratory.

Six of the ten antibodies tested which bind DR beta chains bound to both the transfectants. However CA2, DA2 and M2.2, all of which bind to isolated DR beta chains in Western immunblots, failed to bind to these transfectants, suggesting that they either bind elsewhere in the beta chain or that amino acid residues within the

beta 1 domain form only part of the epitope defined by these antibodies. Localisation of antibody binding sites within molecules is complicated by the fact that many epitopes are comprised of discontinuous amino acids rather than of stretches of linear amino acids. Several formulae exist for predicting the antibody binding epitopes within proteins; these involve the likelihood of occurrences of stretches of amino acids on the surface of proteins, hydrophobicity being one of the criteria used; or as part of a secondary structure such as helix, turn or strand (reviewed in 19). However, none of these formulae are reliable, and they are of little use in predicting discontinuous epitopes. Such sites are easier to predict when the detailed crystallographic structure of the molecule is known. Recently the crystallographic structure for the HLA-A2 molecule was published (20). This, in association with other data, has enabled the putative assignment of of several antibody epitopes (21). To date the crystallographic structure of MHC class II molecules is unkown, although it is postulated that it will be similar to that of the MHC class I molecules (20). Chimaeric murine MHC class II moleculates which have been created by techniques such as exon-shuffling and in vitro mutagenesis (4.22) have been useful in defining both antibody and T cell epitopes on 1A and 1E molecules , as well as the elements of the alpha and beta chains which are important in alpha-beta chain pairing (23).

TDR31.1, M1.6, M2.27, M2.69 and M2.72 all bind to isolated beta chains in Western immunoblots, whereas M1.18 binds to the alpha-beta dimer but not to isolated beta chains. It is possible that the antibodies which bind to isolated beta chains bind to linear rather than discontinuous epitopes. It was found that all of these antibodies bound to both the P815#1 and P815#5 transfected cell lines, rather than binding only to the P815#1 cell line. This indicates that these antibodies are probably

binding to sites within the N-terminal eighty-three amino acids of the DR beta chain as the DR4D115/E^d and $1E^d$ sequences differ significantly from the DR4dw4/E^d sequence for the remainder of the beta 1 domain. Both M2.27 and M2.69 bound more strongly to P815#5 than to P815#1 despite the lower surface expression of class II molecules on the former transfectant. The reason for this is unknown. One of the antibodies tested, X1.26, bound poorly if at all to P815#5 cells, while binding strongly to P815#1 in an ELISA using glutaradehyde fixed cells. It is possible that X1.26 binds to a site which comprises amino acids at the C-terminal end of the the first domain, i.e, between residues 84 and 95. Using in vitro mutagenesis to replace small stretches of the murine $1E^d$ beta gene sequence with the human DR beta gene sequence it is planned to further localise the binding sites of these and other anti-MHC class II antibodies.

Assignments of chains specificity of anti-major histocompatibility complex (MHC) class II antibodies have generally in the past been made by the binding of antibodies to isolated alpha or beta chains in Western immnunoblots (I). However, because of the severe denaturing effect this method has on the class II molecule, it has been difficult to assign chain specificities to many antibodies. More recently, assignment of chain specificity to anti-MHC class II antibodies has been made by the ability of antibodies to bind to mismatched class II alpha and beta chains as the methods for determining this, such as peptide mapping, are difficult to perform. Detailed sequence analysis of mutant murine class II molecules has enabled assignment of some antibody specifities (3). The increased utilisation of genetic engineering techniques has provided other ways for localising antibody binding epitopes within class II molecules (4.5). We have made two hybrid human murine class II molecules which have enabled us to localise the binding of several anti-class II antibodies to the beta 1 domain of DR molecules.

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CLAIMS

1. A process for producing antibodies against a selected target epitope comprising inoculating a host animal with transfected cells syngeneic with the host animal capable of expressing a polypeptide and recovering antibodies from the serum or other body fluid of the animal, the polypeptide comprising a portion comprising the same amino acid sequence as the target epitope and at least one flanking portion having the same amino acid sequence as the corresponding flanking portion of a host animal protein homologous with the protein which bears the target epitope.

2. A process for producing a cell capable of secreting antibodies against a selected target epitope comprising inoculating a host animal with transfected cells syngeneic with the host animal capable of expressing a the polypeptide comprising a portion comprising the same amino acid sequence as the target epitope and at least one flanking portion having the same amino acid sequence as the corresponding flanking portion of a host animal protein homologous with the protein which bears the target epitope and removing antibody-secreting cells from the animal.

3. The process according to claim 1 or claim 2 wherein the host animal and the donor animal from which the transfected cells are derived are substantially genetically identical.

4. A process according to claim 3 wherein the donor and host animals are monozygotic.

5. A process according to any one of claims 1 to 4 wherein the portion of polypeptide comprising the same amino acid sequence as the target epitope comprises a single contiguous protion of at least two amino acid residues in length and up to at least one or more than one domain or parts of more than one domain of the protein

bearing the target epitope.

6. A cell capable of secreting antibodies against a selected polypeptide which cell has been removed from a host animal inoculated with transfected syngeneic cells according to any one of claims 1 to 5.

7. A process for producing an immortal cell capable of secreting antibody against a target epitope comprising immortalising an antibody-secreting animal cell according to claim 6.

8. An immortalised cell, capable of secreting antibodies against a target epitope which cell comprises an immortalised antibody-producing cell according to claim 6 or is a descendant of such a cell.

9. A process for producing monoclonal antibodies against a target epitope comprising culturing an immortalised cell according to claim 6 or a descendant of such a cell, and recovering the monoclonal antibodies.

10. A process for producing monoclonal antibodies against a target epitope of a protein comprising the steps of (a) transfecting cells from a donor animal with a vector containing a DNA sequence encoding for polypeptide comprising a portion having the same amino acid sequence as the target epitope and at least one flanking portion having the same amino acid sequence as the corresponding flanking portion of a host animal protein homologous with the protein which bears the target epitope and capable of expression in the transfected cells,

(b) inoculating an animal syngeneic with the donor animal with the transfectant cells.

(c) fusing immortal cells with cells from the inoculated animal which produce antibody against the target epitope and

(d) culturing the immortal hybrid cells thus produced.

11. A process according to any one of claims 1 to 5, 7, 9 and 10 or a cell according to claim 6 or claim 8 wherein the target epitope is a portion of a human CD4 or HLA protein and the flanking portion of host animal protein is a portion of a non-human homologous cell surface receptor or of a non-human homologous MHC protein respectively and the host animal is a non-human mammal, preferably a mouse.

Fig. 1a.

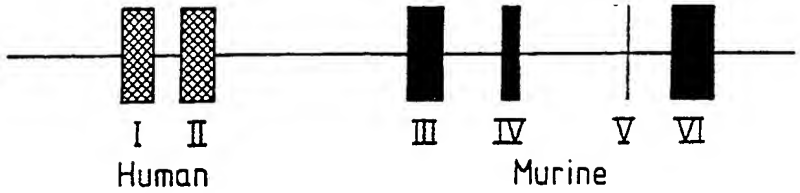


Fig. 1b.

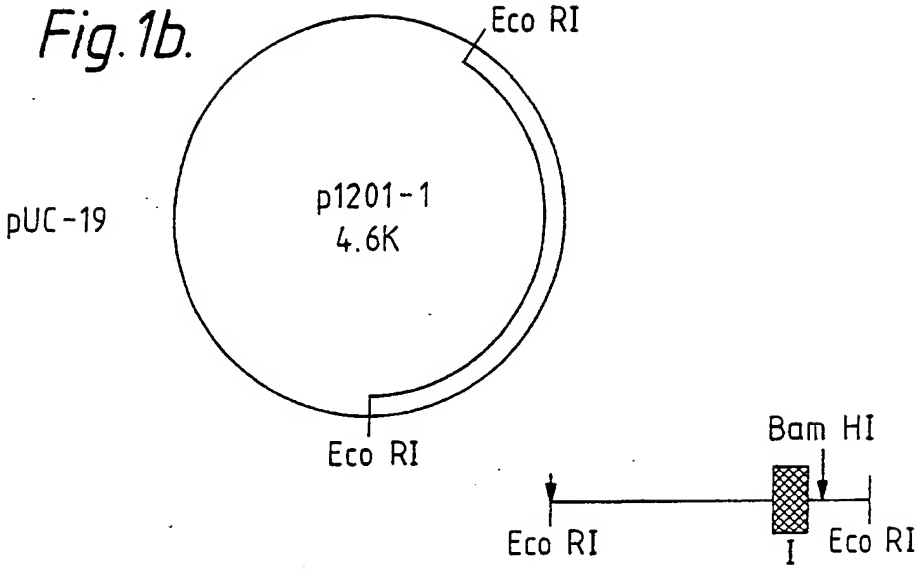


Fig. 1c.

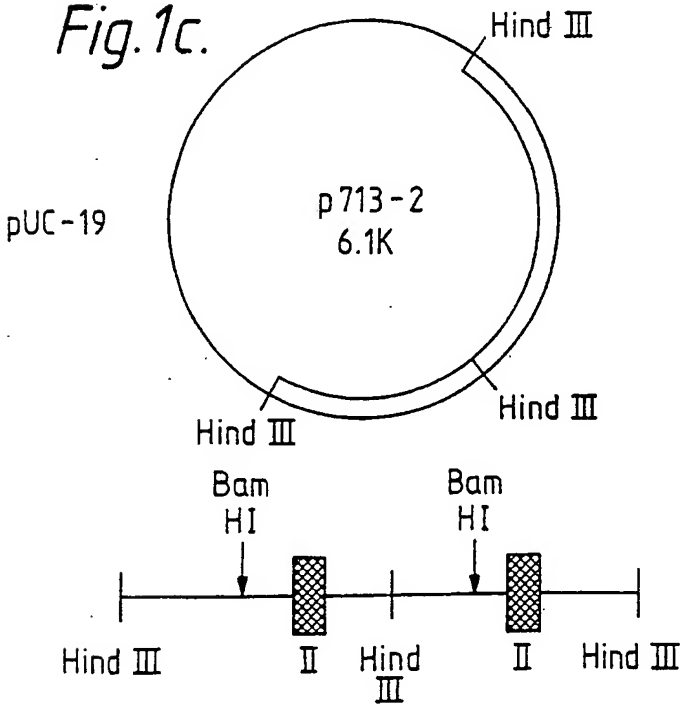
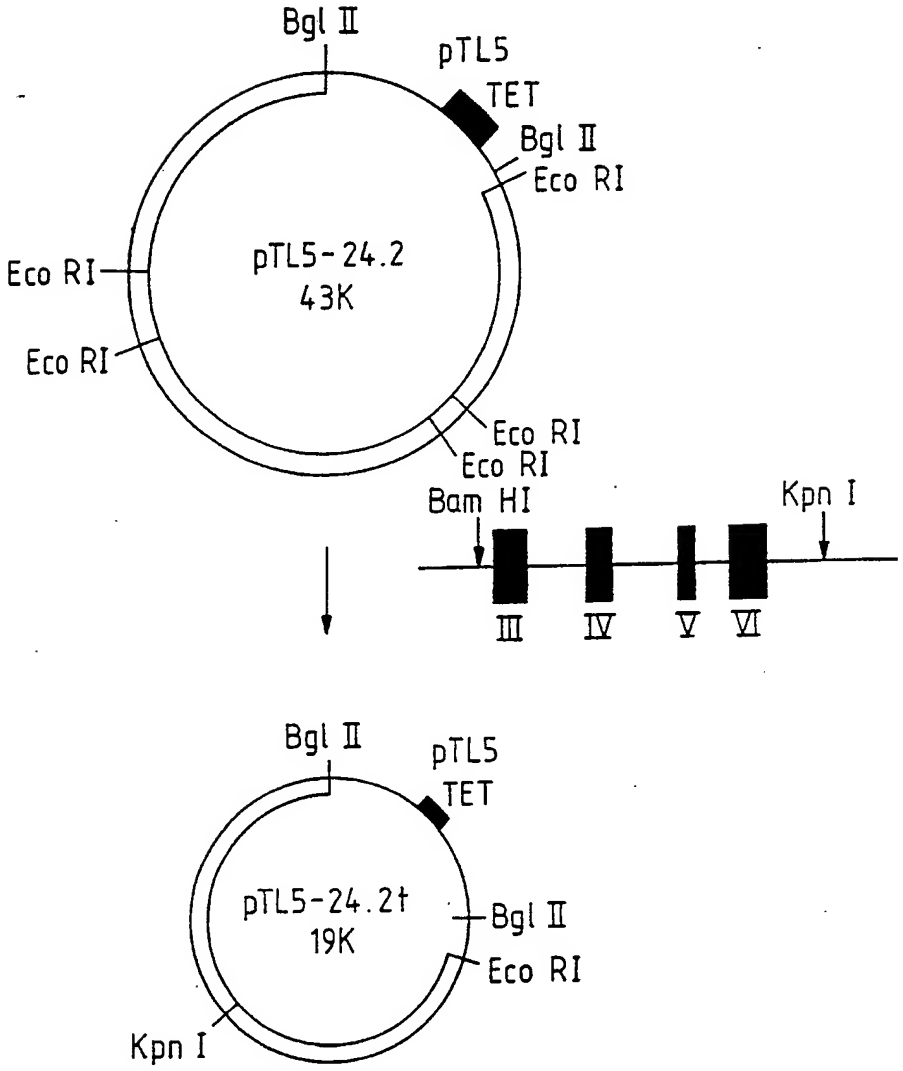


Fig. 1d.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Eco RI
Bam HI
Kpn I
III
IV
V
VI
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TET
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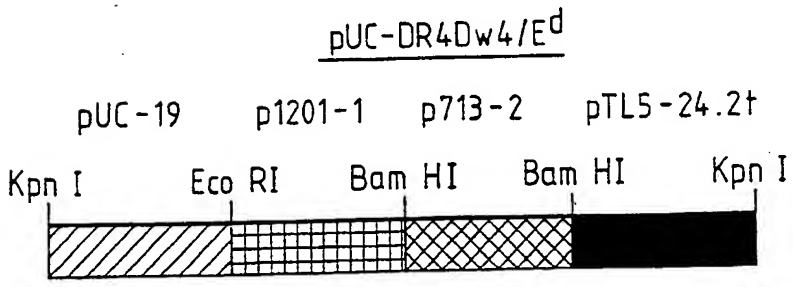
19K
Bgl II
Eco RI
Kpn I

Fig. 1e.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pUC-19
p1201-1
p713-2
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Bam HI
Bam HI
Kpn I

Fig.2.

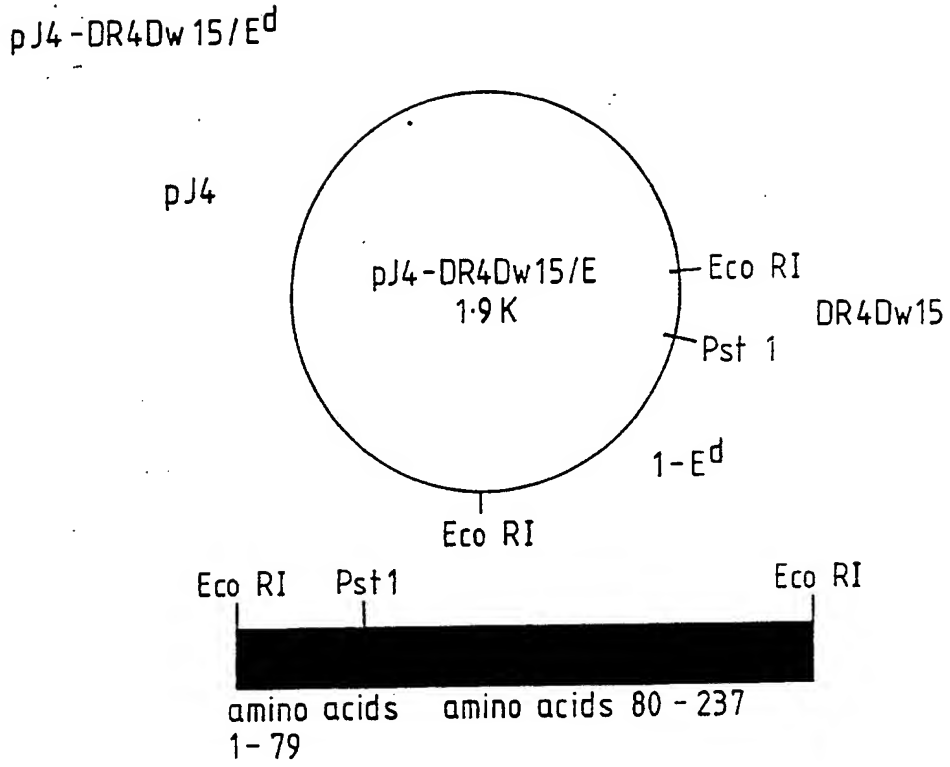

Fig.3.

DR4Dw4/E^d GDTRPRFLEQVKHECHFFNGTERVRFLDRY
DR4DW15/E^d
E VR Y TS Y QH E

DR4Dw4/E^d DSDVGEYRAVTELGRPDAEYWNSQKDLLEQ
DR4Dw15/E^d S
E^d N PEI D

DR4Dw4/E^d KRAAVDTYCRHNYGVGESFTVQRR
DR4Dw15/E^d R EISDK L R
E^d A S EISDK L R

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 88/01013

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 12 P 21/00; C 12 N 5/00; 15/00; //(C 12 P 21/00; C 12 R 1:91); A 61 K 39/395

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	C 12 P; A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	EP, A, 0122814 (B.F. MACH et al.) 24 October 1984 see page 1, paragraph 1; page 6, paragraph 2 - page 7, paragraph 4; page 12, paragraph 5; claims 1,8	1-11

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* Special categories of cited documents: [10]

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
21st March 1989	17. 06. 89
International Searching Authority: EUROPEAN PATENT OFFICE	Signature of Authorized Officer: P.C.G. VAN DER PUTTEN

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 8801013 SA 25268

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 10/04/89
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0122814	24-10-84	None	